First, Kikuchi does not disclose administering a dosage of at least 10 mg/kg to humans. Kikuchi's dosage of 14 mg/kg was discussed for quails, and Kikuchi suggests that only 1/8 of that dosage might be required for humans (see p. 832, first column, first full paragraph.)

Second, Kikuchi does not expressly or inherently disclose that administration of alpha-glucosidase will prolong the life of a human patient with Pompe's disease to at least one year. Kikuchi does not expressly disclose such because he administers alpha glucosidase to quail rather than to human patients with infantile Pompe's disease. Kikuchi does not inherently disclose such because it is not clear from Kikuchi what dosage is to be used in humans. As discussed above, Kikuchi indicates the dosage might be substantially less than that employed in quails. Inherent anticipation cannot be found unless the "prior art *necessarily* functions in accordance with limitations of a process or method claim." (*In re King*, 23 USPQ 136, 138 (Fed. Cir. 1986)). (Emphasis supplied).

De Barcy does not anticipate the present claims, particularly as amended, because De Barcy does not disclose a dosage of at least 10 mg/kg (as recognized by the Examiner in that the rejection was not applied to previous claim 2). De Barcy also does not anticipate in than De Barcy does not disclose that administration prolongs the life of a patient with infantile Pompe's disease by at least one year.

8. Claims 1, 8 and 9-10 stand rejected under 35 USC 102(e) as anticipated by Reuser. The Examiner is requested to reconsider in light of the amendments to the claims. In particular, it is noted that the Examiner did not apply the rejection to previous claim 2 whose element has now been incorporated into claim 1.

9-11. Claims 1-26 stand rejected as obvious over Kikuchi, De Barsy, and Reuser in view of Bijvoet, and Van Hove. Kikuchi, de Barsy and Reuser are cited as disclosing treatment of Pompe's disease by administering human acid alpha-glucosidase. The Examiner acknowledges that the proposed dosages are lower than in certain of the

present claims and that the references do not disclose the feature of gradually increasing the dosage as recited in certain claims. However, the Examiner says that de Barsy suggests increasing the dosage, and Reuser, Bijvoet and Van Hove each disclose endocytosis of the 110 kD form of the enzyme and delivery to the liver and heart upon injection suggesting that the enzyme can be targeted to desired tissues including muscle. The Examiner also says that determining dosages and gradually increasing the dosage would have been a matter of routine observation. The Examiner also comments that the clinical trials described at pp. 37-39 of the present application do not appear to present evidence of surprising results. This rejection is respectfully traversed, particularly as applied to the amended claims.

The Examiner's attention is drawn to the attached Lancet article (Van den Hout et al., Lancet 356, 397 (2000)) that describes the results from one of the clinical trials described in the present application. The results from the trial are remarkable in that all four patients with infantile Pompe's disease survived the 36 weeks of the trial, and past the critical agent of one year by which time most untreated patients have died. The alpha glucosidase activity in muscle approached normal levels, and notable improvements were found in muscle strength and function. Hypertrophic cardiomyopathy (enlargement of the heart) was observed to decrease as were symptoms of cardiac instability. Thus, the trial provides persuasive evidence that treatment with alpha-glucosidase as claimed results in substantial functional improvements of the two organs most affected by infantile Pompe's disease (i.e., muscle and the heart), and prolongs the lives of patients with this disease.

The substantial clinical benefits observed are unexpected viewed from the perspective that the disease has never hitherto been successfully treated and the limited inferences that can be drawn from the cited art. In particular, it was not clear from the cited art to what extent alpha-glucosidase, a protein that is normally produced endogenously, could be taken up by the key target organs in a human when supplied exogenously. It was also unclear to what extent uptake of an exogenous enzyme could

reverse pathology that had already occurred through lack of endogenous enzyme prior to treatment.

The Kikuchi article performs experiments on quails not humans, and only speculates in general terms what might happen in humans. Thus, the reference does not indicate the extent to which humans can take up this enzyme in the heart and muscle tissues. Further, the reference acknowledges that the quail model more closely resembles juvenile or adult Pompe's disease in humans than infantile Pompe's disease in that the quail disease is characterized by residual enzyme and does not include cardiomyopathy or disfunction (p. 831, last paragraph). Thus, the observation of certain beneficial results in quails does not necessarily indicate the clinical outcome in the more challenging circumstances of infantile Pompe's disease in which essentially no enzyme activity is present.

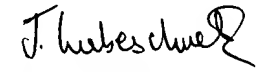
The De Barcy article, which is the only one of the cited references to describe data from administering alpha-glucosidase to humans, reported no clinical benefit, and did not detect uptake except in the liver. Although the reference might indicate that the amount of alpha-glucosidase administered was too small, it is not clear from the reference that this was the only problem, or that increasing the dosage would have achieved a beneficial result. Indeed, Kikuchi's teaching that that the human equivalent of a quail dosage is only one eighth of the quail dosage (p. 832, first column, first full paragraph) might suggest that De Barcy's dosage was not far from being within the right dosage range with the implication that the problem lay elsewhere.

The remaining references discuss uptake of enzyme by cells in vitro or cite to the work of others concerning uptake of enzyme by heart or liver in a guinea pig. These results do not indicate the extent to which alpha glucosidase will be taken up by the heart or muscle in a human infantile Pompe's disease patient, nor whether alpha glucosidase that has been taken up will be able to reverse the damage already caused by lack of the endogenous enzyme, and prolong the life of the patient.

Therefore, it is maintained that the use of dosages in excess of 10 mg/kg patient per week to prolong the life of patients with infantile Pompe's disease, as claimed, was an expected result notwithstanding the cited references.

If the Examiner believes a telephone conference would expedite prosecution of this application, please telephone the undersigned at 650-326-2400.

Respectfully submitted,



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VERSION WITH MARKINGS TO SHOW CHANGES MADE

1. (Amended) A method of treating a human patient with infantile Pompe's disease, comprising: administering to the patient at least 10 mg/kg body weight per week [a therapeutically effective amount] of human acid alpha glucosidase, whereby the patient survives to be at least one year old.

Research letters

Recombinant human α-glucosidase from rabbit milk in Pompe patients

Hannerieke Van den Hout, Arnold J J Reuser, Arnold G Vulto, M Christa B Loonen, Adri Cromme-Dijkhuis, Ans T Van der Ploeg

Pompe's disease is a fatal muscular disorder caused by lysosomal α-glucosidase deficiency. In an open-label study, four babies with characteristic cardiomyopathy were treated with recombinant human α-glucosidase (rhGAA) from rabbit milk at starting doses of 15 mg/kg or 20 mg/kg, and later 40 mg/kg. The enzyme was generally well tolerated. Activity of α-glucosidase normalised in muscle. Tissue morphology and motor and cardiac function improved. The left-ventricular-mass index decreased significantly. We recommend early treatment. Long-term effects are being studied.

Infantile Pompe's disease is a metabolic myopathy with a rapidly progressive course, and is commonly fatal in the first year of life. The disease presents in the first few months after birth with respiratory and feeding difficulties and hypotonia, and hypertrophic cardiomyopathy is characteristic. Major developmental milestones, such as rolling over, sitting, and standing are not achieved.[1] The late-onset form presents as a slowly progressive proximal myopathy. The disease is caused by lysosomal α-glucosidase deficiency and concomitant storage of lysosomal glycogen.[1]

In the development of enzyme therapy for Pompe's disease, production of rhGAA was tested in genetically modified Chinese hamster ovary cells[2,3] and milk of transgenic animals.[4] The two sources seemed suitable, but the high yield in milk and efficacy of the enzyme seen in mice led to large-scale production of rhGAA in transgenic rabbits being chosen.[4] A phase I study of healthy volunteers showed no major side-effects. We report on the first 36 weeks of treatment in patients, during which safety and efficacy data were gathered.

We did a single-centre, open-label pilot study, approved by the institutional review board. Four patients were included with typical symptoms of infantile Pompe's disease (table) and virtual absence of α-glucosidase. We obtained written informed consent from the parents.

RhGAA was administered intravenously once weekly, at starting doses of 20 mg/kg in babies lighter than 6·5 kg (patients 1 and 2) and 15 mg/kg in babies weighing 6·5 kg or more (patients 3 and 4). Doses were increased to 40 mg/kg for all patients. These doses are generally well tolerated without premedication. Adverse events reported were fever, malaise, erythematous rash, sweating, hypoxia, flushing, and tachycardia. The role of IgE-type antibodies in these responses was not evident, but IgG-type antibodies may be relevant. Adverse events were transient and manageable by adaptation of the infusion rate.

α-glucosidase activity in muscle on the starting doses showed a ten-fold increase at 12 weeks of treatment (from 0·15–0·37 nmol/mg per h to 2·1–4·9 nmol/mg per h), but was still lower than normal (8–40 nmol/mg per h). 12 weeks later, with 40 mg/kg RhGAA, α-glucosidase activity was in the normal range for all four patients. On histological assessment, lysosomal glycogen storage was lowered and tissue morphology improved. The total tissue glycogen content did not change significantly.

Skeletal muscle function and strength improved in all patients, most significantly for patient 1, who had the least severe disease at start of treatment. This infant reached milestones that are beyond realistic expectations for a patient with the disease. At 12 months, he could crawl in a four-point position and stand with the support of one arm. Patient 3, who had more severe disease, learned to touch her feet in play. Her improvement has continued, despite producing no endogenous acid α-glucosidase (cross-reactive-immunological-material [CRIM] negative). Patients 2 and 4 also gained strength, most notably in the arms. At start of treatment these two patients (ages 7 months and 8 months) had end-stage disease and muscle function was almost lost. Patient 2 became dependent on a respirator during the inclusion period, as did patient 4, after 10 weeks of treatment, during a bout of pneumonia. The two patients, included before age 3 months, developed normal respiration and became outpatients. All patients showed progress in mental development.

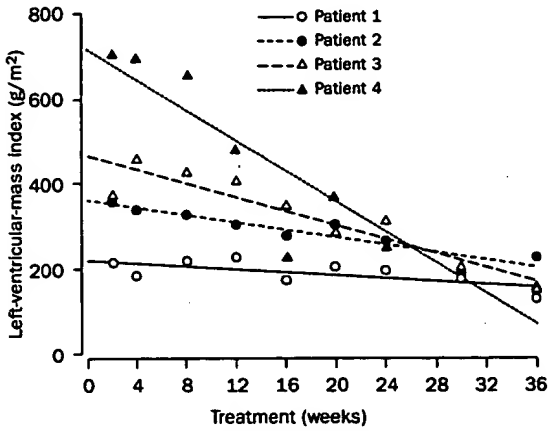
The most prominent effect was on the heart. Left-ventricular posterior-wall thickness and left-ventricular-mass index (figure) decreased in all patients from the start of treatment. Patient 4 responded best. Her left-ventricular-mass index at 36 weeks of treatment was less than 30% of baseline. Symptoms of cardiac instability disappeared in all patients, which was life-saving for patient 4. All patients passed the critical age of 1 year.

RhGAA resulted in uptake of α-glucosidase in skeletal

Patient	Onset of symptoms	Head-lag/ axial hypotonia*	Cardiac hypertrophy/ ECG abnormality	Oxygen need*	Age at diagnosis	Date at inclusion
1	At birth	+	+	−	1 month	3 months
2	3 months	+	+	+	4 months	7 months
3	At birth	+	+	−	14 days	2·5 months
4	3 months	+	+	+	6 months	8 months

ECG=electrocardiography. *During inclusion period.

Patients' characteristics



Linear-regression analyses of left-ventricular-mass index
r=−0·74, r=−0·93, r=−0·93, and r=−0·93 for patients 1, 2, 3, and 4, respectively; each $p<0·05$.

muscle, improved tissue morphology, and stimulated muscle function. The treatment reduced cardiac size, improved cardiac function and clinical condition, and seemed to prolong life. We recommend that treatment be started early. Confirmatory studies in a larger population and long-term follow-up are needed to assess final outcome and quality of life. The milk of transgenic animals seems to be a safe source and opens the way for further exploration of this production method.

The following people played an essential part during the study and should be thought of as researchers: Willem Frans Arts, Wim Hop, Hans de Klerk, Pieter van Doorn, Nynke Weisglas, Barbara Sibbles, Edwin van der Vorrt, Marriet Bruning, Lianne van der Giessen, Hans van Hirtum, and Otto van Diggelen. for other study-related clinical matters, we thank Tom de Vries Lensch, the pharmacy staff, Jan Huijmans, Hans Buller, John van den Anker, and Hans Galjaard, and the research nurses Marielle Etzi, Lyzeth Vendrig, and Angela Franken. Pharming NV, Leiden supported the study.

1 Hirschhorn R. Glycogen storage disease type II: acid α-glucosidase (acid maltase) deficiency. In: Scriver CR, Beaudet AL, Sly WS, Valle D, eds. The metabolic and molecular bases of inherited disease, vol 1. New York: McGraw-Hill, 1995: 2443–64.

2 Fuller M, Van der Ploeg AT, Reuser AJJ, Anson DS, Hopwood JJ. Isolation and characterisation of a recombinant precursor form of lysosomal acid α-glucosidase. *Eur J Biochem* 1995; 234: 903–09.

3 Van Hove JLK, Yang HW, Wu J-Y, Brady RO, Chen YT. High level production of recombinant human lysosomal acid α-glucosidase in Chinese hamster ovary cells which targets to heart muscle and corrects glycogen accumulation in fibroblasts from patients with Pompe disease. *Proc Natl Acad Sci* USA 1996; 93: 65–70.

4 Bijvoet AGA, Van Hirtum H, Kroos MA, et al. Human acid α-glucosidase from rabbit milk has therapeutic effect in mice with glycogen storage disease type II. *Hum Molec Genet* 1999; 8: 2145–53.

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Opioid use in last week of life and implications for end-of-life decision-making

Andrew Thorns, Nigel Sykes

This study was prompted by public and professional concern that the use of opioids for symptom control might shorten life. We retrospectively analysed the pattern of opioid use in the last week of life in 238 consecutive patients who died in a palliative care unit. Median doses of opioid were low (26·4 mg) in the last 24 h of life and patients who received opioid increases at the end of life did not show shorter survival than those who received no increases. The doctrine of double effect therefore need not be invoked to provide symptom control at the end of life.

Concern continues among the general public and health professionals over the use of opioids at the end of life and in particular their possible role in shortening patients' lives.[1] Recent court cases in the UK have raised the possibility that the doctrine of double effect (DDE) is used as a cover for euthanasia.[2] The DDE states that a harmful effect of treatment, even resulting in death, is permissible if it is not intended and occurs as a side-effect of a beneficial action.

We assessed whether symptom control with opioids is associated with shortening of life and how often the DDE is relevant in palliative care. A retrospective case-note review was undertaken of 238 consecutive patients dying in our 62-bed hospice unit. A prospective study might have influenced practice and so was thought inappropriate. The sample proved representative of our patient population, with an median in-patient stay of 9 days (range 1–182) and a mean age of 69 years (SD 13·7).

Time before death (h)	Number of patients	Increase in opioid dose per 24 h		
		>2-fold	1·5–2-fold	>1·5-fold
120–144	161	4 (2·5%)	2 (1·2%)	6 (3·7%)
96–120	170	1 (0·6%)	3 (1·8%)	4 (2·4%)
72–96	188	5 (2·7%)	4 (2·1%)	9 (4·8%)
48–72	207	6 (2·9%)	9 (4·4%)	15 (7·3%)
24–48	222	2 (0·9%)	9 (4·1%)	11 (5·0%)
0–24	239	6 (2·5%)	5 (2·1%)	11 (4·6%)

Table 1: **Patients receiving a greater than 1·5-fold or greater than 2-fold increase in opioid dose per 24 h period before death**

Opioid doses (expressed as parenteral morphine equivalents) were recorded over 24 h during the last week of life and the change in dose calculated for each period. The distribution of at least two-fold increases over the last week of life was analysed, and the characteristics of patients who received a marked increase in opioid dose at the end of life were compared with patients receiving a lesser or no increase. A marked increase was defined as either a greater than 1·5-fold increase in opioid dose in 24 h during the last 48 h of life, when the post-increase dose was greater than 30 mg per 24 h, or a greater than three-fold increase in the last week, during which the increase was greater than 30 mg. We reviewed case notes of the group with marked dose increase to assess whether the DDE could have been included in the decision-making process.

The mean daily opioid dose increased from 42 mg to 55·5 mg (median 15 mg to 26·4 mg), over the last 7 days of life. 212 (89%) patients received opioids in the last 24 h of life, compared with 145 (61%) patients at admission. The proportions of patients requiring a greater than two-fold increase per 24 h period were very small and were evenly distributed over the week (table 1).

Comparison of the patients who received a marked increase in opioids at the end of life with those who received no increase showed no significant difference in survival from admission, frequency of unexpected death, or description of death (table 2). They were, however, significantly more likely to be receiving opioids for pain and were also more likely to be receiving sedatives. The case-note review did not show any cases in which DDE could have been implicated. The reason for the increase was recorded in all cases and the deterioration in condition was noted before the change in dose.

	Marked increase (n=28)	No increase or slight increase (n=210)	p
Mean (SD) length of stay (days)	21 (8·6)	16·4 (3·1)	0·7*
Indication			
Pain	24 (85·7%)	135 (64·3%)	0·01–0·025†
Dyspnoea	9 (32·1%)	63 (30%)	>0·5†
Cough	2 (7·1%)	10 (4·8%)	>0·5†
Death			
Sudden	2 (7·1%)	38 (18·1%)	>0·25†
Gradual	25 (89·3%)	165 (78·6%)	>0·25†
Peaceful	17 (60·7%)	109 (51·9%)	>0·25†
Painful	2 (7·1%)	5 (2·4%)	>0·5†
Restless	6 (21·4%)	25 (11·9%)	>0·25†
Respiratory signs at death	5 (17·9%)	53 (25·2%)	>0·25†
Treatment			
No adjuvant used	12 (42·9%)	112 (53·3%)	>0·25†
No sedative used	0	19 (9%)	<0·05–0·10†

*t test. †χ^2 test.

Table 2: **Characteristics of patients compared by increase in opioid dose**